University of Illinois
at Urbana-Champaign

Department of Agronomy
Turner Hall
1102 South Goodwin Avenue
Urbana, IL 61801

College of Agriculture
217 333-3420

January 19, 1990

Dr. Joshua Lederberg, President
The Rockefeller University
1230 York Avenue
New York, New York 10021-6399

Dear Dr. Lederberg:

This will acknowledge your notes of 10 December 1989 concerning the Kunitz trypsin inhibitor and 'Kunitz' the new soybean cultivar. In addition you asked for information about controversies and other items for your information. My screening of accessions of soybean seed for chemical variants has led to two scientific controversies. One dealt with the Kunitz trypsin inhibitor and the other with seed lectin. None of the below has been published.

1. **Kunitz trypsin inhibitor.** In 1971, my first graduate student, Roger Clark conducted studies on the two major electrophoretic forms of the Kunitz trypsin inhibitor which ultimately were designated as Ti^a and Ti^b. Subsequently, another graduate student located a third form, Ti^c. When plants containing one electrophoretic form were hybridized to plants containing an alternate electrophoretic form, the F_1 seed contained both electrophoretic forms. In the F_2, the electrophoretic forms segregated in a 1:2:1 fashion. Thus, the three Kunitz trypsin inhibitor variants are inherited as codominant alleles in a multiple allelic system at a single locus.

When Roger conducted absorbance and inhibition studies between Ti^a and Ti^b with the same inhibition values (dV_{247}/5 min) had differing absorbances. Furthermore, Ti^a and Ti^b eluates with approximately the same absorbances (A_{280}) had different inhibition values. Roger calculated that the Ti^a forms had different specific activities than the Ti^b forms. He offered the following hypothesis, many amino acid substitutions can occur without changing *in vivo* protein behavior however, some of the substitutions could change *in vitro* properties.

At Roger's final Ph.D. examination, the two plant physiologists on the committee refused to accept his data, refused to sign his Ph.D. papers, and offered their resignations from the committee. The senior plant physiologist even questioned my ability to carry out research. I suspect he believed literally in the one gene-one enzyme hypothesis and didn't understand the concept of variation. As a young faculty member my career was jeopardized by ignorance. The junior plant physiologist was less dogmatic and agreed to oversee Roger's spectrophotometric assays if conducted in his presence.

For two weeks Roger and the faculty member worked like beavers and the results were the same as in Roger's dissertation. Roger received his Ph.D., his manuscript was published in *Biochemical Genetics* (1972) however, he was so bitter about his experience that he left the field of plant genetics. I never again had anything to do with the two plant physiologists.

Recently, Kim *et al.* (1985) conducted amino acid sequence studies on Kunitz trypsin inhibitor Ti^a, Ti^b and Ti^c forms. They found that Ti^c differs from Ti^a in only one amino acid residue, that is, a change from glycine to glutamic acid at residue 55. Ti^b on the other hand, retains glycine at position 55 but differs from Ti^a in at least eight other positions. In addition there are differences in antigenic activity between Ti^a and Ti^b forms.

In the early 1980's I tried to develop a polyclonal antibody test for the Bowman-Birk proteinase inhibitor in soybean seed. I failed because there are several members of this class of inhibitors in soybeans all having similar molecular weights and several cross react with each other. It seemed to me the only reasonable analytical approach was to develop monoclonal antibodies to the Bowman-Birk inhibitor. Alas, I did not have funds and secondly, my department head at that time was a forage physiologist who hadn't the foggiest idea about genetics. I decided that the best tactic was to approach a lab who would be interested in collaborating with me. Fortunately, the USDA Food Safety Research Unit in Berkeley, CA came to my assistance. They developed monoclonals to the Bowman-Birk inhibitor. That is the story behind the printout of the article you enclosed in your note to me.

Last year, I received a small grant from the Illinois Soybean Program Operating Board to screen the USDA soybean collection (13,000 accessions) for Bowman-Birk nulls. All I have is a M.S. graduate assistant, a couple of undergraduate students paid on a hourly basis and limited supply funds. Thus far, we have screened ca. 9000 soybean samples for the absence of the Bowman-Birk inhibitor. About a dozen accessions have been targeted for further study. We sill have some 4000 accessions to screen before initiating genetic studies.

About six months ago, Bob Goldberg published a paper in *The Plant Cell* in which he elucidated the molecular basis for the Kunitz trypsin inhibitor null lines. Enclosed is a photocopy of the article.

Lastly, you posed the question about the "biological function of the inhibitor". The Kunitz trypsin inhibitor in soybean seed most probably does not have a role in the regulation of endogenous proteinases since it only acts as an inhibitor of heterologous proteinases. Its function as a storage

protein is also obscure since during germination it is released from the seed by diffusion or undergoes a limited specific proteolysis. I believe that the function of the Kunitz trypsin inhibitor was to protect wild species against attacks by predators. I am at a loss as to how to conduct controlled experiments to test the hypothesis.

2. **Seed lectin.** In 1974, Bohlool and Schmidt (*Science* 185) concluded that lectin in soybean seed was involved in sites of recognition for rhizobia. Based upon the need to improve our understanding of biological nitrogen fixation NSF, USDA, etc. placed a high priority for funding projects devoted to studying seed lectin.

In late 1975, I was contacted by Steve Pueppke, a biochemical pathologist at Missouri, and he asked me about my thoughts concerning the Bohlool and Schmidt hypothesis. I knew absolutely nothing about seed lectin and thus spent about ten days boning up on lectin chemistry. Immediately, I was dubious about a 120,000 dalton seed lectin having anything to do with rhizobia. I called Steve and agreed to join him in the search for soybeans lacking seed lectin.

I ran pure lectin on an electrophoretic gel to determine its approximate Rf location. Then I had Jim Orf a graduate student go back through our notebooks to check on electrophoretic variants noted in gels. We quickly spotted T-102 as a possible lectin null and informed Steve Pueppke of our information. In the meantime Steve was busy screening selected lines of soybeans I sent to him. In quick order we located five lines that lacked any detectable soybean seed lectin. In all cases my electrophoretic technique agreed with Steve's hemagglutination and fluorescein isothioicyanate cell binding techniques. When the five soybean lines lacking seed lectin were inoculated with *R. japonicum* strains, they nodulated just as well as the control containing seed lectin. To verify our findings, I sent a packet of seeds of a line lacking seed lectin to a biochemist colleague of mine at another institution and asked him to analyze the seed for lectin. He reported back that he could not find any lectin in the seed. Steve and I then prepared a manuscript for *Science.*

I am not in the position to describe precisely as to what happened to the manuscript as Steve was the corresponding author. According to telephone conversations with him, several reviewers rejected the manuscript because of the lack of sensitive analytical procedures, or lack of adequate controls. Other reviewers lauded the manuscript and suggested it be sent to press. After much back and forth letter writing and bickering, the manuscript was approved by the editor and published in *Science* 200:1277 (1978). Several years later I was informed by Steve that the manuscript was reviewed by individuals who had a vested interest in the Bohlool and Schmidt hypothesis. The bickering continued in the literature for several years after the publication of the paper in *Science.* Subsequent genetic

studies revealed that lectin is controlled by dominant *Le* at a single locus. The lectin null lines are homozygous for the recessive allele *le*.

In 1983, Lila Vodkin and Bob Goldberg determined that the lectin gene in *le le* lines are modified by a 3.4 kb insertion showing the structural characteristics of a transposable element (TGM-1 in soybean lectin was the first transposable element described in soybeans). This insertion causes a decrease in transcription reducing the mRNA levels to about 0.01% of the *Le* lines. No soybean lectin polypeptide has been found in seed of *le le* lines. The molecular basis for lectinless seed lines halted all the bickering and the Bohlool and Schmidt hypothesis was put to rest.

Soon afterward using polychonals (I got into the rabbit business) dozens of lectin free seed lines were located in the soybean germplasm collection. Hundreds of lectin free lines were located in *Glycine soja* the wild ancestor of the soybean, *G. max*.

The lessons to be learned from the Kunitz trypsin inhibitor and seed lectin studies are as follows: Academic positions should be filled based upon brainpower, originality, initiative and drive and not based upon where one is born, sex, race, religion or social contacts. The College of Agriculture need to get their act together or they will become the dinosaurs of higher education.

It is imperative that the USDA maintain large collections of our major crops. These collections are not only useful from a practical point of view (plant breeding, sources of pathogen and pest resistance) but also for basic research investigations. After locating nulls for the Kunitz trypsin inhibitor and seed lectin, I elucidated the inheritance of soybean seed lacking or having greatly reduced amounts of lipoxygenase-1, β-amylase and urease. Breaking through the mental black impasse about variation provided the encouragement for colleagues at other institutions in the U.S. and abroad to investigate the variation in other components of soybean seed *e.g.* glycinin, conglycinin, lipoxygenase-2 and -3, etc. In addition, scientists working with other crops have initiated projects to screen germplasm for chemical null variants.

Sincerely yours,

Theodore Hymowitz

Theodore Hymowitz
Professor, Plant Genetics

TH:lrt

Enclosures

P.S. In the mid 1980's I realized that no one had identified individually the soybean chromosomes. Enclosed is a recent paper by Ram Singh revealing such information.